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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification<sup>5</sup> :</b> C07H 15/12, 17/00, A01N 43/04 A61K 31/70, C12Q 1/68 C12P 21/00, 29/06	<b>A1</b>	<b>(11) International Publication Number:</b> WO 93/13114 <b>(43) International Publication Date:</b> 8 July 1993 (08.07.93)
<b>(21) International Application Number:</b> PCT/US92/10785 <b>(22) International Filing Date:</b> 16 December 1992 (16.12.92) <b>(30) Priority data:</b> 07/814,963 24 December 1991 (24.12.91) US <b>(71) Applicant:</b> ISIS PHARMACEUTICALS, INC. [US/US]; 2280 Faraday Avenue, Carlsbad, CA 92008 (US). <b>(72) Inventors:</b> MONIA, Brett, P. ; 4212 Granada Way, Carlsbad, CA 92008 (US). FREIER, Susan, M. ; 2946 Renault Street, San Diego, CA 92122 (US). ECKER, David, J. ; 2609 Colbri Lane, Carlsbad, CA 92009 (US). <b>(74) Agents:</b> CALDWELL, John, W. et al.; Woodcock Washburn Kurtz Mackiewicz & Norris, One Liberty Place 46th Floor, Philadelphia, PA 19103 (US).		<b>(81) Designated States:</b> AU, BB, BG, BR, CA, CS, FI, HU, JP, KP, KR, LK, MG, MN, MW, NO, NZ, PL, RO, RU, SD, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> COMPOSITIONS AND METHODS FOR MODULATING $\beta$ -AMYLOID  <b>(57) Abstract</b>  Compositions and methods are provided for the modulation of expression of the gene encoding $\beta$ -amyloid in both the normal and mutant forms. Oligonucleotides are provided which are specifically hybridizable with RNA or DNA deriving from the $\beta$ -amyloid precursor ( $\beta$ APP) gene encoding $\beta$ -amyloid, having nucleotide units sufficient in identity and number to effect such specific hybridization. Oligonucleotides specifically hybridizable with a translation initiation site or with the codon-717 mutation of $\beta$ APP are provided. Such oligonucleotides can be used for diagnostics as well as for research purposes. Methods are also disclosed for modulating $\beta$ -amyloid expression in cells and tissues using the oligonucleotides provided, and for specific modulation of expression of the mutant $\beta$ APP gene. Methods for diagnosis, detection and treatment of conditions arising from the mutation of the $\beta$ APP gene are also disclosed.		

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-1-

## COMPOSITIONS AND METHODS FOR MODULATING $\beta$ -AMYLOID

### FIELD OF THE INVENTION

This invention relates to the design and synthesis of antisense oligonucleotides which can be administered to modulate the production of  $\beta$ -amyloid protein. These compounds can be used either prophylactically or therapeutically to reduce the severity of disease caused by the abnormal accumulation of  $\beta$ -amyloid. Oligonucleotides which are specifically hybridizable with RNA targets are described.

### BACKGROUND OF THE INVENTION

Alzheimer's disease is the most common cause of dementia during aging in many developed countries. Gradual loss of memory, emotional stability and judgment usually leads to a slow death between four and twelve years after onset. Patients need constant supervision and eventually total custodial care due to the severely debilitated state which results. The cost of diagnosing and managing Alzheimer's patients is currently estimated at more than \$80 billion dollars a year in the US alone. [Selkoe, D.J. (1991) *Scientific American* 265:68-78.]

Two types of brain lesions, senile plaques and neurofibrillary tangles, were described by Dr. Alois Alzheimer in patients with dementia. The senile plaques occur in huge numbers in the areas of the brain responsible for cognitive function, particularly the cerebral cortex, hippocampus and amygdala. These spherical plaques consist of altered neurites (axons and dendrites), which are the long

- 2 -

tapering portions of neurons, surrounding an extracellular mass of filaments. It is presently believed that neurons are caused to degenerate as a result of entanglement in this filament matrix. No treatment is known which either prevents  
5 or retards the progression of the disease.

#### OBJECTS OF THE INVENTION

It is an object of this invention to provide oligonucleotides which are capable of hybridizing with messenger RNA of  $\beta$ -amyloid to modulate the function of the  
10 messenger RNA.

It is a further object to provide oligonucleotides which can modulate the expression of  $\beta$ -amyloid through antisense interaction with messenger RNA.

Yet another object of this invention is to provide  
15 methods of diagnostics and therapeutics for diseases associated with  $\beta$ -amyloid overaccumulation.

Methods, materials and kits for detecting the presence or absence of  $\beta$ -amyloid messenger RNA in a sample suspected of containing it are further objects of the  
20 invention.

Novel oligonucleotides are other objects of the invention.

Another object of the invention is to provide oligonucleotides which are capable of hybridizing selectively  
25 to the messenger RNA of the mutated form of the  $\beta$ -amyloid gene.

Specific inhibition of expression of mutant forms of  $\beta$ -amyloid through hybridization of oligonucleotides with the mutated codon-642 region of the  $\beta$ -amyloid messenger RNA  
30 is yet another object of the invention.

Detection of the mutation from the normal (wild-type) to mutant form of  $\beta$ -amyloid is another object of the invention.

Identification of high-risk conditions based on the  
35 presence of mutant  $\beta$ -amyloid is yet another object of this invention.

- 3 -

A further object of this invention is to provide methods of diagnosis and treatment of conditions arising due to mutation from the wild-type to the mutant form of  $\beta$ -amyloid.

- 5           These and other objects will become apparent to persons of ordinary skill in the art from a review of the instant specification and appended claims.

#### SUMMARY OF THE INVENTION

- In accordance with the present invention,
- 10 oligonucleotides are provided that are specifically hybridizable with DNA or RNA deriving from the gene encoding  $\beta$ -amyloid. The oligonucleotide comprises nucleotide units sufficient in identity and number to effect such specific hybridization. It is preferred that the oligonucleotides be
- 15 specifically hybridizable with the translation initiation codon of the gene, and preferably that the oligonucleotide comprise a sequence CAT. In accordance with another preferred embodiment, oligonucleotides that specifically hybridize with codon 717 of the gene encoding  $\beta$ -amyloid are
- 20 provided. In another such embodiment, oligonucleotides are provided that specifically hybridize preferentially with codon 717 of the gene encoding mutant  $\beta$ -amyloid, preferably comprising a sequence GAT, GAA or GCC. Such oligonucleotides are conveniently and desirably presented in a
- 25 pharmaceutically acceptable carrier.

- In accordance with other preferred embodiments, the oligonucleotides are formulated such that at least some of the linking groups between nucleotide units of the oligonucleotide comprise sulfur-containing species such as
- 30 phosphorothioate moieties.

- Other aspects of the invention are directed to methods for modulating the expression of the gene encoding  $\beta$ -amyloid in cells or tissues and for specifically modulating the expression of the gene encoding mutated  $\beta$ -amyloid in
- 35 cells or tissues suspected of harboring such a mutation. Additional aspects of the invention are directed to methods

- 4 -

of detection of the gene encoding  $\beta$ -amyloid in cells or tissues and specific detection of the gene encoding mutant  $\beta$ -amyloid in cells or tissues suspected of harboring said mutated gene. Such methods comprise contacting cells or  
5 tissues suspected of containing said gene with oligonucleotides in accordance with the invention in order to interfere with the effect of or to detect said gene.

Other aspects of the invention are directed to methods for diagnostics and therapeutics of animals suspected  
10 of having a mutation in the gene encoding  $\beta$ -amyloid. Such methods comprise contacting the animal or cells or tissues or a bodily fluid from the animal with oligonucleotides in accordance with the invention in order to modulate the expression of this gene, to treat conditions arising from  
15 overexpression or mutation of this gene, or to effect a diagnosis thereof.

#### DETAILED DESCRIPTION OF THE INVENTION

The principal constituents of the extracellular filaments at the center of amyloid plaques are not starch, as  
20 the name suggests, but proteins. The identity of the amyloid proteins differs among the various diseases, called amyloidoses, which are characterized by deposits of normal or mutated amyloid protein. Amyloid was first isolated from blood vessels in the meninges of patients with Alzheimer's  
25 disease and was found to be composed of a small protein, named the  $\beta$ -protein. Isolated amyloid cores of senile plaques from the brains of Alzheimer patients were subsequently found to be composed of the same  $\beta$ -protein. This  $\beta$ -amyloid is a small protein approximately 40 amino acids  
30 long, which is derived from the carboxyl terminus of a long (770 amino acids) precursor protein called  $\beta$ -amyloid precursor protein (BAPP). The gene encoding the BAPP protein is located on chromosome 21; this is believed to account for the development of  $\beta$ -amyloid deposits and symptoms of  
35 Alzheimer's disease at an early age in Down syndrome

- 5 -

patients, who have an extra copy of this chromosome.  
[Selkoe, D.J. (1991) *Scientific American*, 265:68-78].

The distinction between normal aging and Alzheimer's disease at the pathological level is largely quantitative rather than qualitative. Most people develop some neurofibrillary tangles and senile plaques by the age of 70 or 80. However, the number of mature plaques and tangles is greater, sometimes by far, in patients with Alzheimer's disease compared to age-matched controls. Diffuse pre-amyloid plaques are found to occur in brain tissue of Alzheimer's disease patients and are actually more abundant than the mature neuritic plaques. Using highly sensitive molecular probes, such diffuse plaques have been detected, not only in the cerebral cortex (the area of the brain involved in cognitive function and implicated in symptoms of dementia) but also in other regions of the brain. Patients with Down's syndrome who die in their teens or twenties, before the inevitable Alzheimer's symptoms appear, have many such diffuse plaques. The deposition of  $\beta$ -amyloid therefore precedes the development of mature plaques with neuron involvement. The abnormal accumulation of  $\beta$ -amyloid is therefore believed to be the cause, rather than a symptom, of Alzheimer's disease. This accumulation can occur through a number of genetic mechanisms.  $\beta$ -amyloid protein deposits have also been found in and around blood vessels of the meninges, skin, intestine and certain other tissues of Alzheimer's disease patients. The process underlying  $\beta$ -amyloid deposition is, therefore, not restricted to the brain. The tendency of these deposits to lie near blood vessels suggests that Alzheimer's disease is similar to certain systemic amyloidoses which are known to have a circulatory origin. Localization of  $\beta$ -amyloid to epithelial cells of the blood vessel walls suggests that the  $\beta$ -amyloid protein that accumulates in the brain may originate in the bloodstream. [Selkoe, D.J. (1991) *Scientific American*, 265:68-78]. Systemic treatment may therefore be possible.

- 6 -

The present invention provides oligonucleotides for modulation of BAPP gene expression.

The hereditary form of Alzheimer's disease known as familial Alzheimer's disease (FAD) is also genetically heterogeneous. Research conducted on families afflicted with FAD has shown that in some families, everyone with Alzheimer's disease has a mutation in codon 717 of the BAPP gene, which is within the  $\beta$ -amyloid peptide region. [Codon 717 (out of 770) is sometimes referred to as codon 642 (out of 695) according to a different transcript numbering system]. Strikingly, while these mutations are not identical at the DNA level, each is a single-base mutation changing amino acid 717 from a valine to another amino acid, such as isoleucine, phenylalanine, or glycine [Goate et al. (1991) *Nature* 349:704-706; Murrell et al. (1991) *Science* 254:97-99; Chartier-Harlin et al. (1991) *Nature* 353:844-846]. The DNA mutations known to cause FAD and their resulting amino acid mutations are shown in Table 1.



- 7 -

TABLE 1

## MUTATIONS AT CODON 717 IN FAMILIAL ALZHEIMER'S DISEASE

Normal:	GTC	ATA	GCG	ACA	GTG	ATC	GTC	ATC	AC
	val	ile	ala	thr	val	ile	val	ile	..
5 Mutations found in	...	...	...	...	...	...	ATC	...	..
Familial Alzheimer's	...	...	...	...	...	...	ile	...	..
Disease (FAD):	...	...	...	...	...	...	TTC	...	..
	...	...	...	...	...	...	phe	...	..
10	...	...	...	...	...	...	GGC	...	..
	...	...	...	...	...	...	gly	...	..

The tight correlation of the codon 717 mutations with Alzheimer's disease in FAD families and the fact that such mutations have not otherwise been found to occur suggest that these mutations are the cause of Alzheimer's disease in these families.  $\beta$ -amyloid overaccumulation can arise, therefore, directly from a mutation in the  $\beta$ APP gene. The present invention provides oligonucleotides which are specifically able to modulate expression of the mutant form of the  $\beta$ APP gene.

The present invention employs oligonucleotides for use in antisense modulation of expression of the  $\beta$ APP gene. In the context of this invention, the term "oligonucleotide" refers to a polynucleotide formed from naturally occurring bases and pentofuranosyl groups joined by native phosphodiester bonds. This term effectively refers to naturally occurring species or synthetic species formed from naturally occurring subunits or their close homologs. The term "oligonucleotide" may also refer to moieties which function similarly to naturally occurring oligonucleotides but which have non-naturally occurring portions. Thus, oligonucleotides may have altered sugar moieties or inter-sugar linkages. Exemplary among these are the phosphorothioate and other sulfur-containing species which

- 8 -

are known for use in the art. In accordance with some preferred embodiments, at least some of the phosphodiester bonds of the oligonucleotide have been substituted with a structure which functions to enhance the ability of the  
5 compositions to penetrate into the region of cells where the RNA or DNA whose activity to be modulated is located. It is preferred that such substitutions comprise phosphorothioate bonds, methyl phosphonate bonds, or short chain alkyl or cycloalkyl structures. In accordance with other preferred  
10 embodiments, the phosphodiester bonds are substituted with other structures which are, at once, substantially non-ionic and non-chiral, or with structures which are chiral and enantiomerically specific. Persons of ordinary skill in the art will be able to select other linkages for use in practice  
15 of the invention.

Oligonucleotides may also include species which include at least some modified base forms. Thus, purines and pyrimidines other than those normally found in nature may be so employed. Similarly, modifications on the pentofuranosyl  
20 portion of the nucleotide subunits may also be effected, as long as the essential tenets of this invention are adhered to. Examples of such modifications are 2'-O-alkyl- and 2'-halogen-substituted nucleotides. Some specific examples of modifications at the 2' position of sugar moieties which are  
25 useful in the present invention are OH, SH, SCH<sub>3</sub>, F, OCH<sub>3</sub>, OCN, O(CH<sub>2</sub>)<sub>n</sub>NH<sub>2</sub> or O(CH<sub>2</sub>)<sub>n</sub>CH<sub>3</sub> where n is from 1 to about 10, and other substituents having similar properties.

Such oligonucleotides are best described as being functionally interchangeable with natural oligonucleotides  
30 (or synthesized oligonucleotides along natural lines), but which have one or more differences from natural structure. All such oligonucleotides are comprehended by this invention so long as they function effectively to hybridize with the BAPP RNA. The oligonucleotides in accordance with this  
35 invention preferably comprise from about 5 to about 50 nucleic acid base units. It is more preferred that such oligonucleotides comprise from about 8 to 25 nucleic acid

- 9 -

base units, and still more preferred to have from about 12 to 25 nucleotide units. As will be appreciated, a nucleotide unit is a base-sugar combination suitably bound to an adjacent nucleotide unit through phosphodiester or other  
5 bonds.

The oligonucleotides used in accordance with this invention may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including Applied  
10 Biosystems (Foster City, CA). Any other means for such synthesis may also be employed, however the actual synthesis of the oligonucleotides are well within the talents of the routineer. It is also well known to use similar techniques to prepare other oligonucleotides such as the  
15 phosphorothioates and alkylated derivatives.

In accordance with this invention, persons of ordinary skill in the art will understand that messenger RNA includes not only the information to encode a protein using the three letter genetic code, but also associated  
20 ribonucleotides which form a region known to such persons as the 5'-untranslated region, the 3'-untranslated region, the 5' cap region and intron/exon junction ribonucleotides. Thus, oligonucleotides may be formulated in accordance with this invention which are targeted wholly or in part to these  
25 associated ribonucleotides as well as to the informational ribonucleotides. In preferred embodiments, the oligonucleotide is specifically hybridizable with a transcription initiation site, a translation initiation site, a 5' cap region, an intron/exon junction, coding sequences or  
30 sequences in the 5'- or 3'-untranslated region.

The oligonucleotides of this invention are designed to be hybridizable with messenger RNA derived from the BAPP gene. Such hybridization, when accomplished, interferes with the normal roles of the messenger RNA to  
35 cause a modulation of its function in the cell. The functions of messenger RNA to be interfered with include all vital functions such as translocation of the RNA to the site.

- 10 -

for protein translation, actual translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and possibly even independent catalytic activity which may be engaged in by the RNA. The overall effect of  
5 such interference with the RNA function is to modulate expression of the BAPP gene.

The oligonucleotides of this invention can be used in diagnostics, therapeutics, prophylaxis, and as research reagents and kits. Since the oligonucleotides of this  
10 invention hybridize to the BAPP gene and its mRNA, sandwich and other assays can easily be constructed to exploit this fact. Furthermore, since the oligonucleotides of this invention hybridize preferentially to the mutant form of the BAPP gene, such assays can be devised for screening of cells  
15 and tissues for BAPP conversion from wild-type to mutant form. Such assays can be utilized for diagnosis of FAD stemming from BAPP gene mutation. Provision of means for detecting hybridization of oligonucleotide with the BAPP gene can routinely be accomplished. Such provision may include  
20 enzyme conjugation, radiolabelling or any other suitable detection systems. Kits for detecting the presence or absence of BAPP or of mutant BAPP may also be prepared.

For therapeutic or prophylactic treatment, oligonucleotides are administered in accordance with this  
25 invention. Oligonucleotides may be formulated in a pharmaceutical composition, which may include carriers, thickeners, diluents, buffers, preservatives, surface active agents and the like in addition to the oligonucleotide. Pharmaceutical compositions may also include one or more  
30 active ingredients such as antimicrobial agents, antiinflammatory agents, anesthetics, and the like in addition to oligonucleotide.

The pharmaceutical composition may be administered in a number of ways depending on whether local or systemic  
35 treatment is desired, and on the area to be treated. Administration may be topically (including ophthalmically, vaginally, rectally, intranasally), orally, by inhalation, or

- 11 -

parenterally, for example by intravenous drip, subcutaneous, intraperitoneal or intramuscular injection.

Formulations for topical administration may include ointments, lotions, creams, gels, drops, suppositories, 5 sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms may also be useful.

Compositions for oral administration include 10 powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders may be desirable.

Formulations for parenteral administration may 15 include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives.

Dosing is dependent on severity and responsiveness of the condition to be treated, but will normally be one or more doses per day, with course of treatment lasting from 20 several days to several months or until a cure is effected or a diminution of disease state is achieved. Persons of ordinary skill can easily determine optimum dosages, dosing methodologies and repetition rates.

The following examples illustrate the present 25 invention and are not intended to limit the same.

- 12 -

**EXAMPLES**Example 1

Synthesis of Oligonucleotides: Unmodified DNA oligonucleotides are synthesized on an automated DNA synthesizer (Applied Biosystems model 380B) using standard phosphoramidite chemistry with oxidation by iodine.  $\beta$ -cyanoethyl-diisopropyl-phosphoramidites are purchased from Applied Biosystems (Foster City, CA). For phosphorothioate oligonucleotides, the standard oxidation bottle is replaced by a 0.2 M solution of 3H-1,2-benzodithiole-3-one 1,1-dioxide in acetonitrile for the stepwise thiation of the phosphite linkages. The thiation cycle wait step is increased to 68 seconds and is followed by the capping step.

2'-O-methyl phosphorothioate oligonucleotides are synthesized using 2'-O-methyl  $\beta$ -cyanoethyl-diisopropyl-phosphoramidites (Chemgenes, Needham MA) and the standard cycle for unmodified oligonucleotides, except the wait step after pulse delivery of tetrazole and base is increased to 360 seconds. The 3'-base used to start the synthesis is a 2'-deoxyribonucleotide.

2'-O-alkylated adenosines and corresponding amidites are prepared as disclosed in PCT patent application Serial Number US91/00243, filed January 11, 1991, which is assigned to the same assignee as the instant application and which is incorporated by reference herein.

After cleavage from the controlled pore glass column (Applied Biosystems) and deblocking in concentrated ammonium hydroxide at 55°C for 18 hours, the oligonucleotides are purified by precipitation twice out of 0.5 M NaCl with 2.5 volumes ethanol. Analytical gel electrophoresis is accomplished in 20% acrylamide, 8 M urea, 45 mM Tris-borate buffer, pH 7.0. Oligodeoxynucleotides and phosphorothioates are judged from electrophoresis to be greater than 80% full length material.

- 13 -

Example 2

BAPP-Luciferase Reporter Gene Assembly: The BAPP-luciferase reporter genes described in this study are assembled using PCR technology. The final construction is assembled in two steps. In the first step, oligonucleotide primers are synthesized for use as primers for PCR cloning of the 5'-regions of  $\beta$ -amyloid. The  $\beta$ -amyloid cDNA template is purchased from the American Type Culture Collection (ATCC number 61910) in Bethesda, MD. The oligonucleotide primers used for this step of the cloning procedure are 5'-ACA-TTA-TGC-TAG-CGC-AGC-GGT-AGG-CGA-GAG-CAC-3' (sense; SEQ ID NO: 24) and 5'-GAG-ATC-TGA-AGC-TTC-GTC-CAG-GCG-GCC-AGC-AGG-A-3' (antisense; SEQ ID NO: 25). These primers are used in standard PCR reactions using the non-mutant  $\beta$ -amyloid cDNA as template. These primers are expected to produce a DNA product of 204 base pairs corresponding to sequences -142 to +48 (relative to the translational initiation site) of the  $\beta$ -amyloid gene, flanked by NheI and HindIII restriction endonuclease sites. This PCR product contains the 5'-nontranslated and translational initiation regions of the BAPP gene. The PCR product is gel purified, precipitated, washed and resuspended in water using standard procedures. This product is then cloned into a steroid-regulatable (Mouse Mammary Tumor Virus promoter) luciferase expression plasmid that we have constructed using the restriction endonucleases NheI and HindIII.

In the second step of the plasmid construction, primers are synthesized for the cloning of the codon-717 (mutation-sensitive) region of the BAPP gene. The oligonucleotide PCR used for this step of the cloning procedure are 5'-GAG-ATC-TGA-AGC-TTG-GTG-CAA-TCA-TTG-GAC-TCA-TG-3' (sense; SEQ ID NO: 26) and 5'-GAG-ATC-TGA-AGC-TTA-CCA-CCC-CTC-AGC-ATC-ACC-AAG-GTG-ATG-AC-3' (antisense; SEQ ID NO: 27). These primers are used in standard PCR reactions using the non-mutant BAPP cDNA as template. These primers are expected to produce a DNA product of 135 base pairs corresponding to sequences +489 to +660 (relative to the

- 14 -

translation initiation site) of the BAPP gene, flanked by HindIII restriction sites. Following purification of the PCR product, the DNA insert is cloned into the construction described in step one of the procedure using the restriction  
5 endonuclease HindIII.

The resulting expression vector encodes a BAPP/luciferase fusion mRNA which is expressed under control of the steroid-inducible MMTV promoter. Translation of this fusion mRNA is dependent on initiation at the BAPP AUG codon.  
10 Furthermore, sequences (ACC-ACC-CCT) which encode the protein-processing recognition sequence Arg-Gly-Gly at the BAPP/luciferase fusion site were incorporated into the step two antisense primer. This sequence is known to be cleaved by specific enzymes following translation to produce the B-  
15 amyloid protein fragment and free luciferase.

#### Example 3

##### Transfection of Cells with Plasmid DNA:

Transfections are performed as described by Greenberg, M.E., in Current Protocols in Molecular Biology, (F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.A. Smith, J.G. Seidman  
20 and K. Strahl, eds.), John Wiley and Sons, NY, with the following modifications. HeLa cells are plated on 60 mm dishes at  $5 \times 10^5$  cells/dish. A total of 10  $\mu$ g of DNA is added to each dish, of which 9  $\mu$ g is BAPP-luciferase reporter  
25 plasmid and 1  $\mu$ g is a vector expressing the rat glucocorticoid receptor under control of the constitutive Rous sarcoma virus (RSV) promoter. Calcium phosphate-DNA coprecipitates are removed after 16-20 hours by washing with Tris-buffered saline [50 mM Tris-Cl (pH 7.5), 150 mM NaCl]  
30 containing 3 mM EGTA. Fresh medium supplemented with 10% fetal bovine serum is then added to the cells. At this time, cells are pre-treated with antisense oligonucleotides prior to activation of reporter gene expression by dexamethasone.



- 15 -

Example 4

Oligonucleotide Treatment of Cells: Immediately following plasmid transfection, cells are washed three times with Opti-MEM (Gibco), prewarmed to 37°C. Two ml of Opti-MEM  
5 containing 10 µg/ml N-[1-(2,3-dioleyloxy)propyl]-N,N,N,-trimethylammonium chloride (DOTMA) (Bethesda Research Labs, Gaithersburg, MD) is added to each dish and oligonucleotides are added directly and incubated for 4 hours at 37°C. Opti-MEM is then removed and replaced with the appropriate cell  
10 growth medium containing oligonucleotide. At this time, reporter gene expression is activated by treatment of cells with dexamethasone to a final concentration of 0.2 µM. Cells are harvested 12-16 hours following steroid treatment.

Example 5

15 Luciferase Assays: Luciferase is extracted from cells by lysis with the detergent Triton X-100, as described by Greenberg, M.E., in Current Protocols in Molecular Biology, (F.M. Ausubel, R. Brent, R.E. Kingston, D.D. Moore, J.A. Smith, J.G. Seidman and K. Strahl, eds.), John Wiley and  
20 Sons, NY. A Dynatech ML1000 luminometer is used to measure peak luminescence upon addition of luciferin (Sigma) to 625 µM. For each extract, luciferase assays are performed multiple times, using differing amounts of extract to ensure that the data are gathered in the linear range of the assay.

25 Example 6

Antisense Oligonucleotide Inhibition of BAPP-Luciferase Gene Expression: A series of antisense phosphorothioate oligonucleotides targeted to the translation initiation codon of BAPP are screened using the BAPP-  
30 luciferase reporter gene system described in the foregoing examples. The base sequences and sequence ID numbers of these oligonucleotides are shown in Table 2.

- 16 -

TABLE 2

<u>SEQ ID NO:</u>	<u>SEQUENCE:</u>
1	GCC AAA CCG GGC AGC ATC GC
5 2	AGC ATC GCG ACC CTG CGC GG
3	AAA CCG GGC AGC ATC GCG AC

Example 7

Antisense inhibition of BAPP-luciferase expression using gapped 2'-O-methyl phosphorothioate oligonucleotides: A series of oligonucleotides are designed having phosphorothioate linkages throughout, and also having 2'-O-methyl nucleotide modifications on at least one base at the 5'- and 3'-termini. The result is a phosphorothioate, 2'-O-methylated oligonucleotide having a "gap" in the 2'-O-methyl modifications (the nucleotides in the "gap" region are 2'-deoxynucleotides). Because 2'-O-methylated nucleotides are resistant to RNAase H cleavage, the deoxy "gap" permits RNAase H cleavage to be directed to this region of the oligonucleotide, and thus to the desired region (here the AUG) of the target mRNA.

These oligonucleotides are shown in Table 3:

TABLE 3

**GAPPED 2'-O-METHYL PHOSPHOROTHIOATE OLIGONUCLEOTIDES  
TARGETED AGAINST THE AUG REGION OF THE BAPP mRNA**

25 (Phosphorothioates throughout; 2'-O-methyl nucleotides shown in **bold**)

<u>SEQ ID NO</u>	<u>SEQUENCE</u>
1	GCC <b>AAA</b> CCG GGC AGC ATC GC
2	AGC ATC GCG ACC CTG <b>CGC</b> GG
30 3	AAA CCG GGC AGC ATC <b>GCG</b> AC

- 17 -

Example 8

Antisense oligonucleotide inhibition of expression of the mutant BAPP-Luciferase gene having a G-to-A mutation at codon 717: A series of antisense phosphorothioate

5 oligonucleotides targeted to codon 717 of the mutant BAPP gene in which a G-to-A mutation at this codon results in a valine-to-isoleucine mutation [Goate et al. (1991) Nature 349:704-706] are screened using the BAPP-luciferase reporter gene system described in the foregoing examples. The base  
10 sequences and sequence ID numbers of these oligonucleotides are shown in Table 4.

TABLE 4

OLIGONUCLEOTIDES TARGETED AGAINST THE G-to-A  
MUTATION OF MUTANT BAPP CODON 717

15	<u>SEQ ID NO</u>	<u>SEQUENCE</u>
	4	GTG ATG ATG ATC ACT
	5	GGT GAT GAT GAT CAC TG
	6	AGG TGA TGA TGA TCA CTG T
	7	AAG GTG ATG ATG ATC ACT GTC

20 Example 9

Antisense inhibition of mutant BAPP-luciferase expression using gapped 2'-O-methyl phosphorothioate oligonucleotides: A series of oligonucleotides are designed

having phosphorothioate linkages throughout, and also having  
25 2'-O-methyl nucleotide modifications on at least one base at the 5'- and 3'-termini. The result is a phosphorothioate, 2'-O-methylated oligonucleotide having a "gap" in the 2'-O-methyl modifications (the nucleotides in the "gap" region are 2'-deoxynucleotides). Because 2'-O-methylated nucleotides  
30 are resistant to RNAase H cleavage, the deoxy "gap" permits RNAase H cleavage to be directed to this region of the oligonucleotide, and thus to the desired region of the target mRNA (here the G-to-A mutation at codon 717).

- 18 -

These oligonucleotides are shown in Table 5:

TABLE 5

GAPPED 2'-O-METHYL PHOSPHOROTHIOATE OLIGONUCLEOTIDES TARGETED AGAINST CODON 717 OF THE MUTANT BAPP mRNA

5 (Phosphorothioates throughout; 2'-O-Methyl nucleotides shown in bold)

<u>SEQ ID NO</u>	<u>SEQUENCE</u>
4	GTG ATG ATG ATC ACT
5	GGT GAT GAT GAT CAC TG
10 6	AGG TGA TGA TGA TCA CTG T
7	AAG GTG ATG ATG ATC ACT GTC

#### Example 10

Antisense oligonucleotide inhibition of expression of the mutant BAPP-Luciferase Gene having a G-to-T mutation at codon 717: A series of antisense phosphorothioate oligonucleotides targeted to codon 717 of the mutant BAPP gene in which a G-to-T mutation at this codon results in a valine-to-phenylalanine mutation [Murrell et al. (1991) *Science* 254:97-99] are screened using the BAPP-luciferase reporter gene system described in the foregoing examples. The base sequences and sequence ID numbers of these oligonucleotides are shown in Table 6.

TABLE 6

OLIGONUCLEOTIDES TARGETED AGAINST THE G-to-A MUTATION OF BAPP

25

<u>SEQ ID NO</u>	<u>SEQUENCE</u>
8	GTG ATG AAG ATC ACT
9	GGT GAT GAA GAT CAC TG
10	AGG TGA TGA AGA TCA CTG T
30 11	AAG GTG ATG AAG ATC ACT GTC

- 19 -

Example 11

Antisense inhibition of mutant BAPP-luciferase expression using gapped 2'-O-methyl phosphorothioate oligonucleotides: A series of oligonucleotides are designed having phosphorothioate linkages throughout, and also having 2'-O-methyl nucleotide modifications on at least one base at the 5'- and 3'-termini. The result is a phosphorothioate, 2'-O-methylated oligonucleotide having a "gap" in the 2'-O-methyl modifications (the nucleotides in the "gap" region are 2'-deoxynucleotides). Because 2'-O-methylated nucleotides are resistant to RNAase H cleavage, the deoxy "gap" permits RNAase H cleavage to be directed to this region of the oligonucleotide, and thus to the desired region of the target mRNA (here the G-to-T mutation at codon 717).

These oligonucleotides are shown in Table 7:

TABLE 7

GAPPED 2'-O-METHYL PHOSPHOROTHIOATE OLIGONUCLEOTIDES TARGETED AGAINST CODON 717 REGION OF MUTANT BAPP mRNA

(Phosphorothioates throughout; 2'-O-Methyl nucleotides shown in bold)

<u>SEQ ID NO</u>	<u>SEQUENCE</u>
8	GTG ATG AAG ATC ACT
9	GGT GAT GAA GAT CAC TG
10	AGG TGA TGA AGA TCA CTG T
25 11	AAG GTG ATG AAG ATC ACT GTC

Example 12

Antisense oligonucleotide inhibition of mutant BAPP-Luciferase Gene Expression: A series of antisense phosphorothioate oligonucleotides targeted to codon 717 of the mutant BAPP gene in which a T-to-G mutation at this codon results in a valine-to-glycine mutation [Chartier-Harlin et al. (1991) *Nature* 353:844-846] are screened using the BAPP-luciferase reporter gene system described in the foregoing examples. The base sequences and sequence ID numbers of these oligonucleotides are shown in Table 8.

- 20 -

TABLE 8  
OLIGONUCLEOTIDES TARGETED AGAINST THE  
T-to-G MUTATION OF BAPP

5	<u>SEQ ID NO</u>	<u>SEQUENCE</u>
	12	GTG ATG CCG ATC ACT
	13	GGT GAT GCC GAT CAC TG
	14	AGG TGA TGC CGA TCA CTG T
	15	AAG GTG ATG CCG ATC ACT GTC

10 Example 13

Antisense inhibition of mutant BAPP-luciferase expression using gapped 2'-O-methyl phosphorothioate oligonucleotides: A series of oligonucleotides are designed having phosphorothioate linkages throughout, and also having 2'-O-methyl nucleotide modifications on at least one base at the 5'- and 3'-termini. The result is a phosphorothioate, 2'-O-methylated oligonucleotide having a "gap" in the 2'-O-methyl modifications (the nucleotides in the "gap" region are 2'-deoxynucleotides). Because 2'-O-methylated nucleotides are resistant to RNAase H cleavage, the deoxy "gap" permits RNAase H cleavage to be directed to this region of the oligonucleotide, and thus to the desired region of the target mRNA (here the T-to-G mutation at codon 717).

These oligonucleotides are shown in Table 9:

- 21 -

TABLE 9

GAPPED 2'-O-METHYL PHOSPHOROTHIOATE OLIGONUCLEOTIDES TARGETED  
AGAINST CODON 717 OF MUTANT BAPP mRNA

(Phosphorothioates throughout; 2'-O-Methyl  
nucleotides shown in bold)

5	<u>SEQ ID NO</u>	<u>SEQUENCE</u>
	12	GTG ATG CCG ATC ACT
	13	GGT GAT GCC GAT CAC TG
	14	AGG TGA TGC CGA TCA CTG T
10	15	AAG GTG ATG CCG ATC ACT GTC

Example 14

Cell culture and treatment with oligonucleotides:

Human umbilical vein endothelial cells (HUVEC) (Clonetics, San Diego CA) are cultured in EGM-UV medium (Clonetics).

- 15 Cells are used between the second and sixth passages. Cells grown in 96-well plates are washed three times with Opti-MEM (GIBCO, Grand Island, NY) prewarmed to 37°C. 100 µl of Opti-MEM containing either 10 µg/ml N-[1-(2,3-dioleyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA, Bethesda Research
- 20 Labs, Bethesda MD) is added to each well. Oligonucleotides are sterilized by centrifugation through 0.2 µm Centrex cellulose acetate filters (Schleicher and Schuell, Keene, NH). Oligonucleotides are added as 20x stock solution to the wells and incubated for 4 hours at 37°C. Medium is removed
- 25 and replaced with 150 µl of the appropriate growth medium containing the indicated concentration of oligonucleotide. The presence of DOTMA during the first 4 hours incubation with oligonucleotide increased the potency of the oligonucleotides at least 100-fold. This increase in potency
- 30 correlated with an increase in cell uptake of the oligonucleotide.

Example 15

ELISA screening of antisense oligonucleotides for activity against BAPP gene expression in HUVEC cells:

- 35 Expression of BAPP by HUVEC cells can be quantitated using specific antibodies (Anti-β-Amyloid, Boehringer Mannheim) in

- 22 -

an ELISA. Cells are grown to confluence in 96 well microtiter plates and gently washed three times with a buffered isotonic solution containing calcium and magnesium such as Dulbecco's phosphate buffered saline (D-PBS). The

5 cells are then directly fixed on the microtiter plate with 1 to 2% paraformaldehyde diluted in D-PBS for 20 minutes at 25°C. The cells are washed again with D-PBS three times. Nonspecific binding sites on the microtiter plate are blocked with 2% bovine serum albumin in D-PBS for 1 hour at 37°C.

10 Cells are incubated with the antibody diluted in blocking solution for 1 hour at 37°C. Unbound antibody is removed by washing the cells three times with D-PBS. Antibody bound to the cells is detected by incubation with a 1:1000 dilution of biotinylated goat anti-mouse IgG (Bethesda Research

15 Laboratories, Gaithersburg, MD) in blocking solution for 1 hour at 37°C. Cells are washed three times with D-PBS and then incubated with a 1:1000 dilution of streptavidin conjugated to  $\beta$ -galactosidase (Bethesda Research Laboratories) for 1 hour at 37°C. The cells are washed three

20 times with D-PBS for 5 minutes each. The amount of  $\beta$ -galactosidase bound to the specific antibody is determined by developing the plate in a solution of 3.3 mM chlorophenolred- $\beta$ -D-galactopyranoside, 50 mM sodium phosphate, 1.5 mM  $MgCl_2$ ; pH=7.2 for 2 to 15 minutes at 37°C. The concentration of the

25 product is determined by measuring the absorbance at 575 nm in an ELISA microtiter plate reader.



- 23 -

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Monia et al.
- 5 (ii) TITLE OF INVENTION: Composition and Methods for  
Modulating  $\beta$ -Amyloid
- (iii) NUMBER OF SEQUENCES: 27
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Woodcock Washburn Kurtz  
Mackiewicz & Norris
  - 10 (B) STREET: One Liberty Place - 46th Floor
  - (C) CITY: Philadelphia
  - (D) STATE: PA
  - (E) COUNTRY: USA
  - (F) ZIP: 19103
- 15 (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: DISKETTE, 3.5 INCH, 1.44 Mb STORAGE
  - (B) COMPUTER: IBM PS/2
  - (C) OPERATING SYSTEM: PC-DOS
  - (D) SOFTWARE: WORDPERFECT 5.0
- 20 (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER: n/a
  - (B) FILING DATE: herewith
  - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
  - 25 (A) APPLICATION NUMBER:
  - (B) FILING DATE:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Jane Massey Licata

- 24 -

(B) REGISTRATION NUMBER: 32,257

(C) REFERENCE/DOCKET NUMBER: ISIS-0480

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (215) 568-3100

5 (B) TELEFAX: (215) 568-3439

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

10 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GCCAAACCGG GCAGCATCGC 20

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

20 (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

AGCATCGCGA CCCTGCGCGG 20

(2) INFORMATION FOR SEQ ID NO: 3:

25 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

- 25 -

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

AAACCGGGCA GCATCGCGAC 20

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

10 (D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GTGATGATGA TCACT 15

(2) INFORMATION FOR SEQ ID NO: 5:

15 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

20 (iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GGTGATGATG ATCACTG 17

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 19

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

- 26 -

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

AGGTGATGAT GATCACTGT 19

(2) INFORMATION FOR SEQ ID NO: 7:

5 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10 (iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

AAGGTGATGA TGATCACTGT C 21

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 15

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GTGATGAAGA TCACT 15

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17

25 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

- 27 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GGTGATGAAG ATCACTG 17

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 19  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

AGGTGATGAA GATCACTGT 19

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 21  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

20 AAGGTGATGA AGATCACTGT C 21

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 15  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

- 28 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

GTGATGCCGA TCACT 15

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 17  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

GGTGATGCCG ATCACTG 17

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 19  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

20 AGGTGATGCC GATCACTGT 19

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 21  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

- 29 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

AAGGTGATGC CGATCACTGT C 21

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 15  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GTGATGACGA TCACT 15

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17  
15 (B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

20 GGTGATGACG ATCACTG 17

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19  
(B) TYPE: nucleic acid  
25 (C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

- 30 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

AGGTGATGAC GATCACTGT 19

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 21  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

AAGGTGATGA CGATCACTGT C 21

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 15  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

20 GTGATGNNGA TCACT 15

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 17  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes



- 31 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

GGTGATGNNG ATCACTG 17

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 19  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

AGGTGATGNN GATCACTGT 19

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 21  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iv) ANTI-SENSE: yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

20 AAGGTGATGN NGATCACTGT C 21

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 33  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iv) ANTI-SENSE: no

- 32 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

ACATTATGCT AGCGCAGCGG TAGGCGAGAG CAC 33

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 34  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iv) ANTI-SENSE: no

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

GAGATCTGAA GCTTCGTCCA GGCGGCCAGC AGGA 34

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 35  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

20 GAGATCTGAA GCTTGGTGCA ATCATTGGAC TCATG 35

- 33 -

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 44

(B) TYPE: nucleic acid

5 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(iv) ANTI-SENSE: no

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

GAGATCTGAA GCTTACCACC CCTCAGCATC ACCAAGGTGA TGAC 44

- 34 -

## CLAIMS

What is claimed is:

1. An oligonucleotide comprising from 8 to 25 nucleotide units specifically hybridizable with selected DNA or RNA deriving from the  $\beta$ APP gene.
2. The oligonucleotide of claim 1 specifically hybridizable with a translation initiation site or codon 717 of the  $\beta$ APP gene.
3. The oligonucleotide of claim 1 in a pharmaceutically acceptable carrier.
4. The oligonucleotide of claim 1 wherein at least one of the linking groups between nucleotide units of the oligonucleotide comprises a sulfur-containing species.
5. The oligonucleotide of claim 4 wherein said sulfur-containing species comprises a phosphorothioate.
6. The oligonucleotide of claim 1 wherein at least one of the nucleotides is modified at the 2' position.
7. The oligonucleotide of claim 6 wherein the modification is a 2'-O-alkyl.
8. An oligonucleotide specifically hybridizable with selected DNA or RNA deriving from the  $\beta$ APP gene and comprising one of the sequences:  
5'.....3'  
25 GTG ATG NNG ATC ACT (SEQ ID NO: 20)  
GGT GAT GNN GAT CAC TG (SEQ ID NO: 21)  
AGG TGA TGN NGA TCA CTG T (SEQ ID NO: 22)  
AAG GTG ATG NNG ATC ACT GTC (SEQ ID NO: 23)
9. The oligonucleotide of claim 8 in a pharmaceutically acceptable carrier.
10. The oligonucleotide of claim 8 wherein at least one of the linking groups between nucleotide units of the oligonucleotide comprises a sulfur-containing species.
11. The oligonucleotide of claim 10 wherein said sulfur-containing species comprises a phosphorothioate.

- 35 -

12. The oligonucleotide of claim 8 wherein at least one of the nucleotides is modified at the 2' position.

13. The oligonucleotide of claim 12 wherein the 5 nucleotide modification is a 2'-O-alkyl.

14. An oligonucleotide specifically hybridizable with selected DNA or RNA deriving from the BAPP gene and comprising one of the sequences:

5'.....3'

10 GCC AAA CCG GGC AGC ATC GC (SEQ ID NO: 1)  
AGC ATC GCG ACC CTG CGC GG (SEQ ID NO: 2)  
AAA CCG GGC AGC ATC GCG AC (SEQ ID NO: 3)  
GTG ATG ATG ATC ACT (SEQ ID NO: 4)  
GGT GAT GAT GAT CAC TG (SEQ ID NO: 5)

15 AGG TGA TGA TGA TCA CTG T (SEQ ID NO: 6)  
AAG GTG ATG ATG ATC ACT GTC (SEQ ID NO: 7)  
GTG ATG AAG ATC ACT (SEQ ID NO: 8)  
GGT GAT GAA GAT CAC TG (SEQ ID NO: 9)  
AGG TGA TGA AGA TCA CTG T (SEQ ID NO: 10)

20 AAG GTG ATG AAG ATC ACT GTC (SEQ ID NO: 11)  
GTG ATG CCG ATC ACT (SEQ ID NO: 12)  
GGT GAT GCC GAT CAC TG (SEQ ID NO: 13)  
AGG TGA TGC CGA TCA CTG T (SEQ ID NO: 14)  
AAG GTG ATG CCG ATC ACT GTC (SEQ ID NO: 15)

25 15. The oligonucleotide of claim 14 in a pharmaceutically acceptable carrier.

16. The oligonucleotide of claim 14 wherein at least one of the linking groups between nucleotide units of the oligonucleotide comprises a sulfur-containing species.

30 17. The oligonucleotide of claim 16 wherein said sulfur-containing species comprises a phosphorothioate.

18. The oligonucleotide of claim 14 wherein at least one of the nucleotides is modified at the 2' position.

35 19. The oligonucleotide of claim 18 wherein the nucleotide modification is a 2'-O-alkyl.

- 36 -

20. A method of modulating the expression of the BAPP gene comprising contacting tissues or cells containing the gene with an oligonucleotide comprising from 8 to 25 nucleotide units specifically hybridizable with selected DNA or RNA deriving from the BAPP gene.

21. The method of claim 20 wherein said oligonucleotide is specifically hybridizable with a translation initiation site or codon 717 of the BAPP gene.

22. The method of claim 20 wherein said oligonucleotide comprises one of the sequences:

5'.....3'  
 GTG ATG NNG ATC ACT (SEQ ID NO: 20)  
 GGT GAT GNN GAT CAC TG (SEQ ID NO: 21)  
 AGG TGA TGN NGA TCA CTG T (SEQ ID NO: 22)  
 AAG GTG ATG NNG ATC ACT GTC (SEQ ID NO: 23)

23. The method of claim 20 wherein said oligonucleotide comprises one of the sequences:

5'.....3'  
 GCC AAA CCG GGC AGC ATC GC (SEQ ID NO: 1)  
 AGC ATC GCG ACC CTG CGC GG (SEQ ID NO: 2)  
 AAA CCG GGC AGC ATC GCG AC (SEQ ID NO: 3)  
 GTG ATG ATG ATC ACT (SEQ ID NO: 4)  
 GGT GAT GAT GAT CAC TG (SEQ ID NO: 5)  
 AGG TGA TGA TGA TCA CTG T (SEQ ID NO: 6)  
 AAG GTG ATG ATG ATC ACT GTC (SEQ ID NO: 7)  
 GTG ATG AAG ATC ACT (SEQ ID NO: 8)  
 GGT GAT GAA GAT CAC TG (SEQ ID NO: 9)  
 AGG TGA TGA AGA TCA CTG T (SEQ ID NO: 10)  
 AAG GTG ATG AAG ATC ACT GTC (SEQ ID NO: 11)  
 GTG ATG CCG ATC ACT (SEQ ID NO: 12)  
 GGT GAT GCC GAT CAC TG (SEQ ID NO: 13)  
 AGG TGA TGC CGA TCA CTG T (SEQ ID NO: 14)  
 AAG GTG ATG CCG ATC ACT GTC (SEQ ID NO: 15)

24. The method of claim 20 wherein at least one of the linking groups between nucleotide units of the oligonucleotide comprises a sulfur-containing species.

- 37 -

25. The method of claim 24 wherein said sulfur-containing species comprises a phosphorothioate.

26. The method of claim 20 wherein at least one of the nucleotides of the oligonucleotide is modified at the 2' position.

27. The method of claim 26 wherein the nucleotide modification is a 2'-O-alkyl.

28. A method of detecting the presence of the BAPP gene in cells or tissues comprising contacting the cells or tissues with an oligonucleotide comprising from 8 to 25 nucleotide units specifically hybridizable with selected DNA or RNA deriving from the BAPP gene.

29. The method of claim 28 wherein said oligonucleotide is specifically hybridizable with a translation initiation site or codon 717 of the BAPP gene.

30. The method of claim 28 wherein said oligonucleotide comprises one of the sequences:

5'.....3'

GTG ATG NNG ATC ACT (SEQ ID NO: 20)

20 GGT GAT GNN GAT CAC TG (SEQ ID NO: 21)

AGG TGA TGN NGA TCA CTG T (SEQ ID NO: 22)

AAG GTG ATG NNG ATC ACT GTC (SEQ ID NO: 23)

31. The method of claim 28 wherein said oligonucleotide comprises one of the sequences:

25 5'.....3'

GCC AAA CCG GGC AGC ATC GC (SEQ ID NO: 1)

AGC ATC GCG ACC CTG CGC GG (SEQ ID NO: 2)

AAA CCG GGC AGC ATC GCG AC (SEQ ID NO: 3)

GTG ATG ATG ATC ACT (SEQ ID NO: 4)

30 GGT GAT GAT GAT CAC TG (SEQ ID NO: 5)

AGG TGA TGA TGA TCA CTG T (SEQ ID NO: 6)

AAG GTG ATG ATG ATC ACT GTC (SEQ ID NO: 7)

GTG ATG AAG ATC ACT (SEQ ID NO: 8)

GGT GAT GAA GAT CAC TG (SEQ ID NO: 9)

35 AGG TGA TGA AGA TCA CTG T (SEQ ID NO: 10)

AAG GTG ATG AAG ATC ACT GTC (SEQ ID NO: 11)

GTG ATG CCG ATC ACT (SEQ ID NO: 12)

- 38 -

GGT GAT GCC GAT CAC TG (SEQ ID NO: 13)

AGG TGA TGC CGA TCA CTG T (SEQ ID NO: 14)

AAG GTG ATG CCG ATC ACT GTC (SEQ ID NO: 15)

32. The method of claim 28 wherein at least one  
5 of the linking groups between nucleotide units of the  
oligonucleotide comprises a sulfur-containing species.

33. The method of claim 32 wherein said sulfur-  
containing species comprises a phosphorothioate.

34. The method of claim 28 wherein at least one  
10 of the nucleotides of the oligonucleotide is modified at  
the 2' position.

35. The method of claim 34 wherein the  
nucleotide modification is a 2'-O-alkyl.

36. A method of detecting mutant BAPP based on  
15 the differential affinity of particular oligonucleotides  
for mutant vs. wild-type BAPP comprising contacting cells  
or tissues suspected of containing it with one of the  
oligonucleotides:

5'.....3'

20 GTG ATG ACG ATC ACT (SEQ ID NO: 16)

GGT GAT GAC GAT CAC TG (SEQ ID NO: 17)

AGG TGA TGA CGA TCA CTG T (SEQ ID NO: 18)

AAG GTG ATG ACG ATC ACT GTC (SEQ ID NO: 19)

and contacting an identical sample of cells or tissues with  
25 one of the oligonucleotides:

5'.....3'

GTG ATG ATG ATC ACT (SEQ ID NO: 4)

GGT GAT GAT GAT CAC TG (SEQ ID NO: 5)

AGG TGA TGA TGA TCA CTG T (SEQ ID NO: 6)

30 AAG GTG ATG ATG ATC ACT GTC (SEQ ID NO: 7)

GTG ATG AAG ATC ACT (SEQ ID NO: 8)

GGT GAT GAA GAT CAC TG (SEQ ID NO: 9)

AGG TGA TGA AGA TCA CTG T (SEQ ID NO: 10)

AAG GTG ATG AAG ATC ACT GTC (SEQ ID NO: 11)

35 GTG ATG CCG ATC ACT (SEQ ID NO: 12)

GGT GAT GCC GAT CAC TG (SEQ ID NO: 13)

AGG TGA TGC CGA TCA CTG T (SEQ ID NO: 14)



- 39 -

AAG GTG ATG CCG ATC ACT GTC (SEQ ID NO: 15)

37. The method of claim 36 wherein at least one of the linking groups between nucleotide units of at least one of the oligonucleotides comprises a sulfur-containing 5 species.

38. The method of claim 37 wherein said sulfur-containing species comprises a phosphorothioate.

39. The method of claim 36 wherein at least one of the nucleotides of the oligonucleotide is modified at 10 the 2' position.

40. The method of claim 39 wherein the nucleotide modification is a 2'-O-alkyl.

41. A method of treating conditions arising from overproduction of  $\beta$ -amyloid comprising contacting an animal 15 with an oligonucleotide comprising from 8 to 25 nucleotide units specifically hybridizable with selected DNA or RNA deriving from the  $\beta$ APP gene.

42. The method of claim 41 wherein said oligonucleotide comprises one of the sequences:

20 5'.....3'  
GTG ATG NNG ATC ACT (SEQ ID NO: 20)  
GGT GAT GNN GAT CAC TG (SEQ ID NO: 21)  
AGG TGA TGN NGA TCA CTG T (SEQ ID NO: 22)  
AAG GTG ATG NNG ATC ACT GTC (SEQ ID NO: 23)

25 43. The method of claim 41 wherein said oligonucleotide comprises one of the sequences:

30 5'.....3'  
GCC AAA CCG GGC AGC ATC GC (SEQ ID NO: 1)  
AGC ATC GCG ACC CTG CGC GG (SEQ ID NO: 2)  
AAA CCG GGC AGC ATC GCG AC (SEQ ID NO: 3)  
GTG ATG ATG ATC ACT (SEQ ID NO: 4)  
GGT GAT GAT GAT CAC TG (SEQ ID NO: 5)  
AGG TGA TGA TGA TCA CTG T (SEQ ID NO: 6)  
AAG GTG ATG ATG ATC ACT GTC (SEQ ID NO: 7)  
35 GTG ATG AAG ATC ACT (SEQ ID NO: 8)  
GGT GAT GAA GAT CAC TG (SEQ ID NO: 9)  
AGG TGA TGA AGA TCA CTG T (SEQ ID NO: 10)

- 40 -

AAG GTG ATG AAG ATC ACT GTC (SEQ ID NO: 11)

GTG ATG CCG ATC ACT (SEQ ID NO: 12)

GGT GAT GCC GAT CAC TG (SEQ ID NO: 13)

AGG TGA TGC CGA TCA CTG T (SEQ ID NO: 14)

5 AAG GTG ATG CCG ATC ACT GTC (SEQ ID NO: 15)

44. The method of claim 41 wherein at least one of the linking groups between nucleotide units of the oligonucleotide comprises a sulfur-containing species.

45. The method of claim 44 wherein said sulfur-  
10 containing species comprises a phosphorothioate.

46. The method of claim 41 wherein at least one of the nucleotides of the oligonucleotide is modified at the 2' position.

47. The method of claim 46 wherein the  
15 nucleotide modification is a 2'-O-alkyl.

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US92/10785

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) : Please See Extra Sheet.

US CL : 536/23.1, 24.1, 24.31, 24.33, 24.5; 435/69.1, 6; 514/44

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1, 24.1, 24.31, 24.33, 24.5; 435/69.1, 6; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
<u>X</u> Y	Science, Vol. 254, issued 04 October 1991, Murrell et al., "A Mutation in the Amyloid Precursor Protein Associated with Hereditary Alzheimer's Disease" pages 97-99, abstract.	<u>1-2, 8-14</u> 3-7,9-13,15-47
<u>X</u> Y	Nature, Vol. 349, issued 21 February 1991, Goate et al., "Segregation of a Missense Mutation in the Amyloid Precursor Protein Gene with Familial Alzheimer's Disease", pages 704-706, abstract.	<u>1-2, 8, 14</u> 3-7,9-13,15-47
A	US, A, 4,581,333 (Kourilsky et al.) 08 April 1986, column 1, lines 15-21.	28-40
A	Nucleic Acids and Molecular Biology, Vol. 1, 1987 (Springer-Verlag, Berlin), Houba-Herlin et al., "Antisense RNA", pages 210-220, pages 211-214.	20-27, 41-47

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be part of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"A" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

10 MARCH 1993

Date of mailing of the international search report

31 MAR 1993

Name and mailing address of the ISA/US  
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Form PCT/ISA/210 (second sheet)(July 1992)\*

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US92/10785

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

(Telephone Practice)

Group 1, claims 1-27, drawn to oligonucleotides and their method of use to block expression. Classified in class 536 subclass 24.5 and class 435, subclass 69.1.

Group 2, claims 28-40, drawn to an assay for the presence of a gene. Classified in class 435, subclass 6.

Group 3, claims 41-47, drawn to methods of human treatment, classified in class 514, subclass 44.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US92/10785

## A. CLASSIFICATION OF SUBJECT MATTER:

IPC (5):

C12Q1/68

C07H 15/12, 17/00; A01N 43/04; A61K 31/70; G12-1/68; C12P 21/00, 29/06